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MINIREVIEW

Resolving Nonstop Translation Complexes Is a Matter of Life or Death

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Problems during gene expression can result in a ribosome that has translated to the 3′ end of an mRNA without terminating at a stop codon, forming a nonstop translation complex. The nonstop translation complex contains a ribosome with the mRNA and peptidyl-tRNA engaged, but because there is no codon in the A site, the ribosome cannot elongate or terminate the nascent chain. Recent work has illuminated the importance of resolving these nonstop complexes in bacteria. Transfer-messenger RNA (tmRNA)-SmpB specifically recognizes and resolves nonstop translation complexes in a reaction known as *trans*-translation. *trans*-Translation releases the ribosome and promotes degradation of the incomplete nascent polypeptide and problematic mRNA. tmRNA and SmpB have been found in all bacteria and are essential in some species. However, other bacteria can live without *trans*-translation because they have one of the alternative release factors, ArfA or ArfB. ArfA recruits RF2 to nonstop translation complexes to promote hydrolysis of the peptidyl-tRNAs. ArfB recognizes nonstop translation complexes in a manner similar to tmRNA-SmpB recognition and directly hydrolyzes the peptidyl-tRNAs to release the stalled ribosomes. Genetic studies indicate that most or all species require at least one mechanism to resolve nonstop translation complexes. Consistent with such a requirement, small molecules that inhibit resolution of nonstop translation complexes have broad-spectrum antibacterial activity. These results suggest that resolving nonstop translation complexes is a matter of life or death for bacteria.

Bacteria perform transcription and translation in the same cellular compartment because they do not have nuclei. One advantage to this arrangement is that bacteria can rapidly respond to environmental challenges by producing new proteins. The time between transcription of a gene and the availability of the corresponding protein is minimized because the mRNA does not have to be processed or exported, and translation of an mRNA can initiate before transcription is complete. However, using a single compartment for transcription and translation has serious consequences for protein quality control because there are limited opportunities for mRNA proofreading. Mechanisms used by eukaryotes to ensure that the mRNA is intact are generally absent in bacteria. For example, in eukaryotes, 3′ polyadenylation is used as a signal that the mRNA transcript is complete. This signal is read at several steps, including nuclear export and translation initiation, which requires interaction between poly(A)-binding proteins and translation initiation factors (1, 2). In contrast, the bacterial ribosome does not require any information from the 3′ end of the mRNA to initiate translation, so there is no assurance that the mRNA is complete or intact (3). mRNAs can be truncated by many events, including premature termination of transcription, nuclease activity, and physical damage. As a consequence, bacterial ribosomes frequently translate mRNAs that do not have a stop codon ("nonstop" mRNAs). When a ribosome reaches the 3′ end of a nonstop mRNA, it is trapped in a nonstop translation complex. In this complex, the mRNA and peptidyl-tRNA in the P site prevent dissociation of the ribosome, but the complex cannot elongate or terminate because there is no codon in the A site. A nonstop complex can also be formed when a ribosome stalls during translation and the mRNA is cleaved in the A site (4–6). Estimates from *Escherichia coli* suggest that 2% to 4% of translation reactions end in a nonstop translation complex (7). At that rate, an average ribosome is involved in ~5 nonstop translation complexes per cell division cycle. Clearly, the protein synthesis capacity of the cell would be severely compromised if these complexes could not be quickly resolved. To cope with the prevalence of nonstop translation complexes, bacteria have a remarkable mechanism known as *trans*-translation, which can release the ribosome and target the nonstop mRNA and nascent polypeptide for rapid degradation.

RESOLUTION OF NONSTOP TRANSLATION COMPLEXES BY tmRNA-SmpB

trans-Translation is performed by a ribonucleoprotein complex consisting of transfer-messenger RNA (tmRNA), a specialized RNA molecule, and SmpB, a small protein. tmRNA has elements of both a tRNA and an mRNA. The 5′ and 3′ ends of tmRNA form a structure resembling the acceptor arm and TΨC arm of alanyl-tRNA (8, 9). The remainder of tmRNA includes several pseudoknots and a specialized reading frame that is decoded during *trans*-translation (8, 10–12). SmpB binds tightly with tmRNA and completes the tRNA-like structure by mimicking the anticodon stem (13–15). The acceptor arm of tmRNA is charged with alanine by alanyl-tRNA synthetase and bound by EF-Tu in the same manner as $tRNA^{Ala}$ (8, 16, 17). During *trans*-translation, tmRNA-SmpB specifically recognizes a nonstop translation complex and is accommodated in the ribosomal A site (Fig. 1) (18–21). The nascent polypeptide is transferred to the alanine charged to tmRNA, and SmpB-tmRNA is translocated to the P site. During translocation, a large swivel of the 30S head of the ribosome allows the reading frame of tmRNA to enter the mRNA channel (22). The first codon of the tmRNA reading frame is aligned in the A site, and translation resumes using the tmRNA reading frame as a mes-

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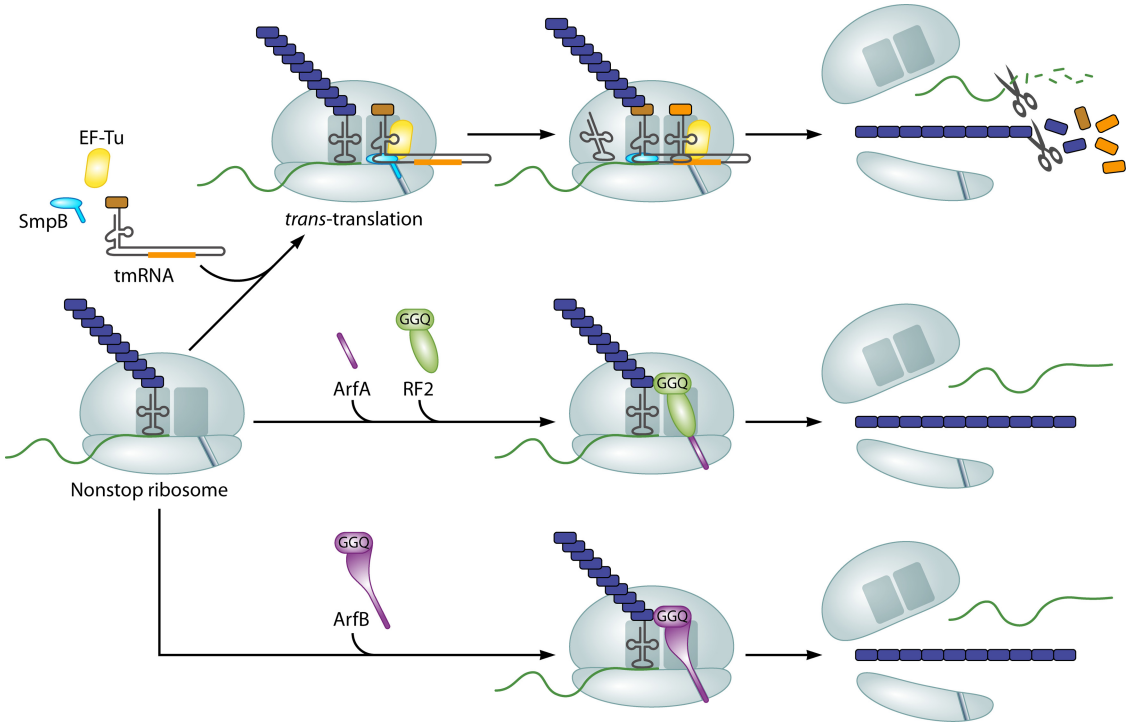

FIG 1 Mechanisms for resolving nonstop translation complexes. During *trans*-translation (top), tmRNA-SmpB recognizes nonstop translation complexes by binding in the empty mRNA channel and uses a reading frame within tmRNA to mediate the release of the ribosome and target the nascent polypeptide for proteolysis. The problematic mRNA is also degraded. Some bacteria have backup systems that use either ArfA or ArfB to recognize nonstop translation complexes. ArfA recruits RF2, which uses its GGQ motif to hydrolyze the peptidyl-tRNA in the ribosome. It is not known how ArfA recognizes nonstop translation complexes, but it might bind in the empty mRNA channel in a manner similar to that of SmpB and ArfB binding. ArfB contains a GGQ motif and directly hydrolyzes the peptidyl-tRNA on the ribosome. ArfA and ArfB release the ribosome but do not target the nascent polypeptide for degradation. See the text for details.

sage. Correct alignment of tmRNA in the mRNA channel requires sequence-specific contacts between tmRNA and SmpB (23). Translation of the tmRNA reading frame terminates at a stop codon, releasing the ribosome and a protein that includes the tmRNA-encoded peptide tag at the C terminus (24). The peptide tag is recognized by multiple proteases in the cell, ensuring that the protein is rapidly degraded (24–28). The nonstop mRNA is also targeted for degradation during *trans*-translation (29–31). Thus, the overall effect of the reaction is to remove the problematic mRNA and the incomplete protein and to release the ribosome (Fig. 1).

A crystal structure from Neubauer et al. captures an early step of *trans*-translation and shows how tmRNA-SmpB recognizes nonstop translation complexes (32) (Fig. 2). In the structure, the

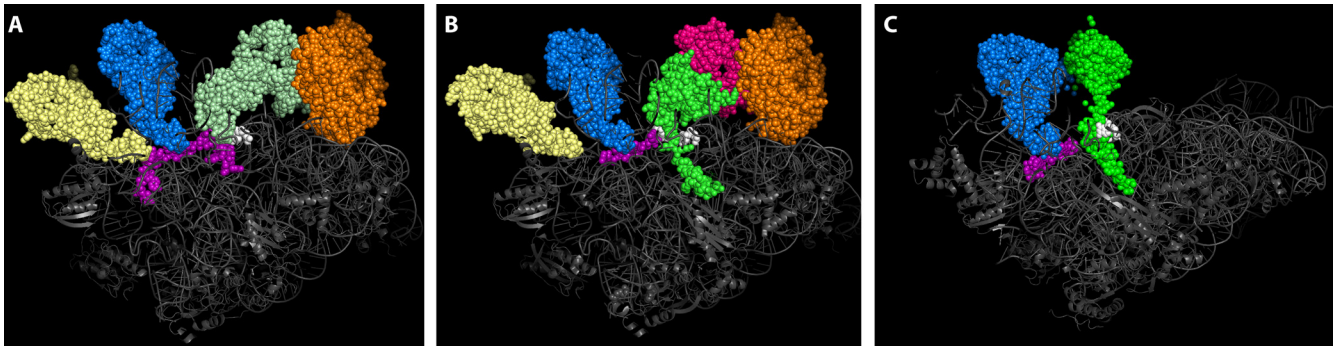

FIG 2 Recognition of nonstop translation complexes. Structure models of an elongation complex (A) with an intact mRNA compared to recognition of nonstop translation complexes by tmRNA-SmpB (B) and ArfB (C) are shown. The 30S ribosomal subunits are shown in gray, with decoding nucleotides G530, A1492, and A1493 in white. (A) An elongation complex trapped by kirromycin from PDB 2WRQ, with mRNA (purple), E-site tRNA (yellow), P-site tRNA (blue), and A-site tRNA (green) bound with EF-Tu (orange). (B) *trans*-Translation complex trapped by kirromycin from PDB 4ABR. The tRNA-like domain of tmRNA (pink) bound with EF-Tu (orange) is in an orientation similar to that of the acceptor stem of the tRNA shown in panel A. SmpB (green) occupies the codon-anticodon region and extends into the empty mRNA channel. (C) In nonstop translation complexes recognized by ArfB (from PDB 4DH9), ArfB (green) extends into the empty mRNA channel, with the catalytic GGQ domain near the peptidyl-tRNA in the P site (blue).

tRNA-like domain of tmRNA, bound with SmpB and EF-Tu, is trapped in the A site of a nonstop translation complex during accommodation using the drug kirromycin. Overall, the structure resembles an elongation complex with tmRNA-SmpB in place of the acylated tRNA. The acceptor arm of tmRNA is in the same orientation as the acceptor arm of the acylated tRNA, and SmpB takes the place of the anticodon stem. However, SmpB also makes contacts in the decoding center and empty mRNA channel that appear to mimic the missing mRNA. The 16S rRNA residues A1492, A1493, and G530, which interact with the mRNA in an elongation complex, directly contact SmpB in the nonstop complex. In addition, the C terminus of SmpB forms a helix that extends into the empty mRNA channel between the decoding center and the leading edge of the ribosome. Chemical footprinting and mutational studies support the hypothesis of the presence of these interactions during *trans*-translation (33, 34). This crystal structure suggests that tmRNA-SmpB could not be accommodated in elongating ribosomes because the mRNA would obstruct SmpB interactions with the 16S rRNA (Fig. 2). Consistent with this model, competition experiments show that tmRNA-SmpB does not interfere with translation elongation or termination *in vivo* (35).

Whereas the crystal structure suggests that the mRNA channel downstream of the A site must be empty for tmRNA-SmpB to bind, kinetic data indicate that the mRNA channel does not always have to be empty for *trans*-translation to occur. The rate of *trans*-translation *in vitro* was measured using ribosomes stalled on mRNAs of different lengths (36). When the ribosomes were stalled with the mRNA channel completely occupied (with >15 nucleotides downstream of the P site), the reaction was extremely slow, consistent with the mRNA blocking tmRNA-SmpB. However, the reaction was rapid when the ribosomes were stalled with 0 to 6 nucleotides of mRNA downstream of the P site and was inhibited only partially with 9 to 12 nucleotides downstream of the P site. These results imply that mRNA in the A site, and even several codons downstream of the A site, does not interfere with *trans*-translation. The substrates used for the kinetic measurements were generated by omitting a tRNA from the reaction, so they probably do not occur frequently *in vivo*. However, the issue of whether tmRNA-SmpB can act on ribosomes with mRNA extending past the A site has important implications for the mechanism of *trans*-translation. It is possible that the interactions between SmpB and 16S rRNA observed in the crystal structure represent the lowest energy conformation, but these interactions are not required for tmRNA-SmpB to initiate *trans*-translation. Alternatively, when a ribosome stalls on an mRNA that does not completely fill the mRNA channel, it might undergo a structural change that allows SmpB access to the 16S rRNA. For example, the 3′ end of the mRNA might loop out of the A site, or the ribosome could slide to the 3′ end of the mRNA, leaving the A site empty. Such rearrangements could be facilitated by communication between the mRNA channel and the decoding center of the ribosome. Further biochemical experiments are required to determine whether *trans*-translation always requires an empty mRNA channel.

SUBSTRATES FOR *trans*-TRANSLATION

Some of the known substrates for *trans*-translation are consistent with nonstop translation complexes generated by mRNA damage, but others suggest nonrandom or intentional mRNA cleavage to target translation reactions to *trans*-translation. Truncation of mRNA by premature termination of transcription, damage to the mRNA, or 3′-5′ exonucleolytic mRNA turnover would be expected to be largely random and should produce nonstop translation complexes at a variety of positions along many mRNAs. Two proteomic-analysis-scale studies identified proteins tagged by *trans*-translation in *Caulobacter crescentus* and *Francisella tularensis*. Both studies found that many proteins are tagged and that tagging occurs at locations throughout the protein sequence, as would be expected for activity on damaged mRNAs (37, 38).

On the other hand, investigation of *E. coli* proteins that are tagged with high frequency indicates that there are some sequences prone to generation of nonstop translation complexes (39). For example, in some substrates, tagging occurs with high frequency after runs of rare codons or highly inefficient translation termination sequences (40–42). The mRNA is initially complete in these cases, but ribosome stalling during translation elongation or termination exposes the downstream mRNA to exonucleases, which chew back the mRNA to the leading edge of the ribosome to generate substrates for *trans*-translation (4, 43–45). Exonuclease activity by RNase II can promote cleavage of the mRNA in the A site through an unknown mechanism, but RNase II and the corresponding A-site cleavage are not essential for *trans*-translation on known substrates (46, 47). Redundant nuclease activities may ensure that translation complexes stalled for an extended time are targeted for resolution by *trans*-translation.

In addition to ribosome stalling, errors during translation can lead to *trans*-translation. Suppressor tRNAs and drugs that promote miscoding increase the number of proteins tagged by *trans*-translation, demonstrating that readthrough of the stop codon and frameshifting can result in nonstop translation complexes when there is not an in-frame stop codon downstream (48, 49). The examples described above all result in nonproductive translation complexes, which could sequester ribosomes and limit new protein synthesis. The main purpose of *trans*-translation on these substrates is likely to be release of the ribosomes to maintain protein synthesis capacity.

There is also evidence that *trans*-translation is used to ensure the quality of the protein pool. *trans*-Translation increases on large proteins when *dnaK* is deleted, suggesting that misfolding of the nascent polypeptide might trigger mRNA cleavage to target the nascent polypeptide for proteolysis (50). It is now clear that interactions of the nascent chain in the peptide exit tunnel and communication between ribosome-associated chaperones and the catalytic center of the ribosome can affect the rate of translation (51, 52). Terminally misfolded nascent proteins might be targeted to *trans*-translation to ensure that they are rapidly degraded. It is not yet known whether there is a dedicated pathway for generating nonstop complexes that is triggered by misfolding or whether misfolding slows elongation enough to expose the mRNA to nonspecific exonuclease activity.

Finally, *trans*-translation is used intentionally as part of several regulatory circuits. RNase toxin components of toxin-antitoxin systems such as RelE and MazF cut most mRNAs in the cell, generating a large number of nonstop mRNAs and nonstop translation complexes (53, 54). Toxin activity is used to induce stasis, allowing the cell to conserve resources during severe stress (53, 54). Toxins are also activated in a small percentage of cells under optimal growth conditions to generate persister cells that can survive sudden stresses (55). *E. coli* mutants lacking *trans*-translation

activity are defective in recovery from toxin-induced stasis, indicating that resolution of the nonstop translation complexes resulting from toxin activity is important for resuming growth after severe nutritional stress or persistence (56, 57). Individual proteins are also targeted for *trans*-translation through truncation of the cognate mRNAs. Nuclease cleavage sites or transcriptional terminators 5′ of the stop codon have been found in some *arfA* and *kinA* genes (58–60). Translation of these genes results in proteins that are rapidly degraded unless *trans*-translation is impaired, making the encoded protein activity dependent on the state of *trans*-translation. The *arfA* example is described in more detail below. *trans*-Translation is used by LacI in *E. coli* to prevent excess protein accumulation (61). At high concentrations, LacI binds within the 3′ end of its own gene. LacI binding to this site blocks transcription elongation and generates a nonstop mRNA, thereby targeting all newly expressed LacI for proteolysis. The use of *trans*-translation in regulatory circuits may be important for individual species or behaviors, but the evolutionary conservation of *trans*-translation is almost certainly due to the ability to maintain the protein synthesis capacity of the cell.

PHYSIOLOGY OF AND ALTERNATIVES TO *trans*-TRANSLATION

Genes encoding tmRNA (*ssrA*) and SmpB (*smpB*) have been identified in all sequenced bacterial species, including those with severely reduced genomes (62). This conservation suggests that *trans*-translation confers a selective advantage in all environments that support bacterial life. In fact, tmRNA and SmpB have been shown to be essential in several species, including *Neisseria gonorrhoeae*, *Shigella flexneri*, *Helicobacter pylori*, and *Mycobacterium tuberculosis* (63–66). Saturating genome-wide mutagenesis experiments suggest that tmRNA and SmpB are also required for viability in *Haemophilus influenzae*, *Mycoplasma genitalium*, and *Staphylococcus aureus* (67–69). In other bacteria, tmRNA can be deleted with widely varying consequences. In some species, phenotypes of mutants lacking *trans*-translation activity are severe, including defects in virulence (*Salmonella enterica*, *Yersinia pestis*, *Francisella tularensis*, and *Streptococcus pneumoniae*), symbiosis (*Bradyrhizobium japonicum*), and cell cycle control (*C. crescentus*) (38, 70–75). However, *E. coli* and *Bacillus subtilis* mutants that lack *trans*-translation have relatively mild phenotypes, such as increased antibiotic susceptibility and stress response defects (48, 76–78). Recent discoveries have shown that most or all species that do not require *trans*-translation have backup systems that resolve nonstop translation complexes when *trans*-translation activity is not available.

ArfA

On the basis of the evolutionary conservation of *trans*-translation and the differences in phenotypes between *E. coli* and species in which tmRNA is essential, Chadani and coworkers performed a screen for genes that are essential in strains deleted for *ssrA* (79). They identified a single gene, *arfA*, and showed that the ArfA protein can promote hydrolysis of peptidyl-tRNA on nonstop translation complexes in an *in vitro* translation reaction. Release of the ribosomes by ArfA requires RF2, suggesting that ArfA recognizes the empty mRNA channel and recruits RF2 to hydrolyze the peptidyl-tRNA (Fig. 1) (80). However, it is not yet clear how ArfA recognizes nonstop translation complexes.

ArfA is a true backup system for *trans*-translation in that it is active only when *trans*-translation activity is not available. The *arfA* mRNA in *E. coli* includes a cleavage site for RNase III before the stop codon and is efficiently cut by RNase III to produce a nonstop mRNA (58). Translation of *arfA* when *trans*-translation is active results in a tagged ArfA protein that is rapidly degraded. When *ssrA* is deleted, stable and active ArfA protein is produced. Presumably, regulation by *trans*-translation allows ArfA to release nonstop complexes only under physiological conditions where *trans*-translation is inactive or saturated. Most *arfA* genes from other species encode the RNase III cleavage site, but some use a transcriptional terminator before the stop codon to produce a nonstop mRNA (60). Thus, regulation of ArfA by *trans*-translation is conserved even though the mechanism for producing the nonstop mRNA is not.

Genetic experiments with *arfA* suggest that release of ribosomes from nonstop translation complexes is essential in *E. coli* and related species. In *E. coli*, deletion of *arfA* and *ssrA* is synthetically lethal (79). In contrast, *ssrA* is essential in *S. flexneri*, which does not have *arfA*, but *ssrA* can be deleted in *S. flexneri* cells that are engineered to express *E. coli arfA* (64). *arfA* genes have been identified in only a subset of beta- and gammaproteobacteria and a few other species (60). However, the small size of *arfA* makes bioinformatic identification in distantly related bacteria difficult. The presence of *arfA* does not ensure that *trans*-translation is dispensable. *N. gonorrhoeae* has an *arfA* gene, and yet *trans*-translation is essential. The *N. gonorrhoeae arfA* is active when expressed in *E. coli* (60), so either *arfA* is not expressed in *N. gonorrhoeae* or its activity is not sufficient to support viability in the absence of *trans*-translation.

ArfB

A second alternative system, ArfB, was discovered in a multicopy suppressor screen for genes that allowed *E. coli* to survive without tmRNA or ArfA (Fig. 1) (81). Peptidyl-tRNA hydrolase (Pth) activity had been predicted for ArfB on the basis of the presence of a GGQ motif common to release factors and peptidyl-tRNA hydrolases (82). In fact, purified ArfB specifically hydrolyzes peptidyl-tRNA in nonstop translation complexes *in vitro* (81, 83). Structural studies show that ArfB recognizes nonstop complexes in a manner similar to that of SmpB-tmRNA: a C-terminal helix of ArfB extends into the empty mRNA channel, and residues in this helix make contacts with 16S rRNA that are important for activity (Fig. 2) (84, 85). The physiological role of ArfB in *E. coli* is not clear. The chromosomal copy of a*rfB* will not support growth of *E. coli* in the absence of tmRNA and ArfA, and *ssrA* is essential in *S. flexneri* even though *arfB* is present (64, 79). Either ArfB is reserved for special conditions in these species or the availability of ArfA has made ArfB redundant and control of its expression has been lost. In contrast, ArfB in *Caulobacter crescentus* is functional in its chromosomal context and allows cells to survive without *trans*-translation. The *C. crescentus arfB* gene was identified in transposon sequencing (Tn-Seq) experiments as a gene that is essential in cells lacking *ssrA* but not in wild-type cells (H. A. Feaga and K. C. Keiler, unpublished data). ArfB homologs are widely distributed throughout bacterial species. No regulation of ArfB by *trans*-translation has been identified, so, unlike ArfA, ArfB may provide a constitutive, low level of resolution activity that becomes significant only when *trans*-translation is saturated or inactivated.

Mitochondria also have an ArfB homolog, which is named

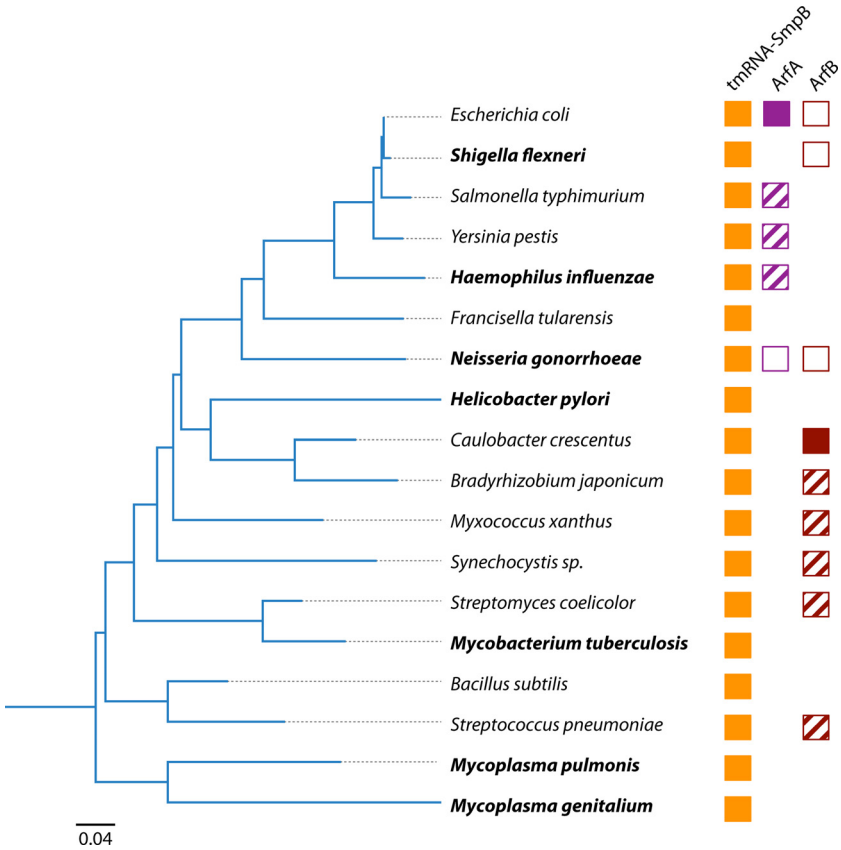

FIG 3 Phylogenetic distribution of *trans*-translation, ArfA, and ArfB. Species in which the phenotype of deleting *ssrA* or *smpB* is known are shown on a phylogenetic tree based on 16S rRNA sequences. Bold names indicate species in which *ssrA* or *smpB* is essential. The presence of genes encoding tmRNA-SmpB, ArfA, and ArfB is shown. For ArfA and ArfB, a filled box indicates that the system is sufficient to maintain viability in the absence of tmRNA-SmpB, an empty box indicates that the system is not sufficient to maintain viability in the absence of tmRNA-SmpB, and a hashed box indicates that it is not yet known whether the system is sufficient to maintain viability. *Salmonella typhimurium*, *Salmonella enterica* serovar Typhimurium.

ICT1 (85, 87). ICT1 hydrolyzes peptidyl-tRNA on the ribosome, and this activity is essential for human cells (87). ArfB and ICT1 both contain an N-terminal GGQ motif and a C-terminal $R(X_3)K(X_6)K(X_2)R$ motif that are required for peptidyl-tRNA hydrolase activity (85). As in bacteria, transcription and translation are performed in a single compartment in mitochondria, so ICT1 may serve to release nonstop complexes and maintain protein synthesis capacity in these organelles. tmRNA has been identified in organelles of some primitive eukaryotes but is not retained in metazoans (62, 88). It appears that most eukaryotic mitochondria kept ArfB and dispensed with *trans*-translation, whereas all bacteria retained *trans*-translation.

The discoveries of ArfA and ArfB have important implications for understanding the role of *trans*-translation and the consequences of nonstop translation complexes. With the exception of *B. subtilis* and *F. tularensis*, all species in which *ssrA* or *smpB* has been deleted encode either ArfA or ArfB (Fig. 3). Moreover, in all cases that have been tested, the ArfA or ArfB backup system becomes essential when *ssrA* is deleted. Therefore, at least one mechanism to resolve nonstop complexes may be required for viability in most or all bacteria. Investigation of unknown alternative resolution mechanisms in *B. subtilis* and *F. tularensis* would test how universal this requirement is. Some nonstop translation complexes may be resolved by "drop-off," dissociation of the peptidyl-tRNA from the ribosome followed by hydrolysis of the free peptidyl-tRNA by peptidyl-tRNA hydrolase (Pth). Drop-off occurs with some nascent chains of two to five amino acids, but longer chains have not been shown to dissociate without prior peptidyl-tRNA hydrolysis within the ribosome (89, 90). Interactions between the nascent polypeptide and the exit channel may prevent drop-off in most cases. The discoveries of ArfA and ArfB make it clear that drop-off alone cannot support viability for most species in the absence of *trans*-translation.

Why is it that all bacteria use *trans*-translation to resolve nonstop complexes, and some use only *trans*-translation, but none use only ArfA or ArfB? ArfA and ArfB do not completely mimic *trans*-translation, because they do not directly target the nascent polypeptide for proteolysis. Presumably, incomplete proteins released by ArfA or ArfB activity must be recognized and degraded by other proteolytic pathways in the cell. The fate of the mRNA during ArfA and ArfB activity is not yet known. It is likely that *trans*-translation is the preferred pathway because it promotes degradation of the incomplete proteins and damaged mRNAs from nonstop complexes in addition to releasing the stalled ribosomes.

TARGETING *trans*-TRANSLATION FOR ANTIBIOTICS

The *trans*-translation pathway is an attractive target for development of new antibiotics because it is required for viability or vir-

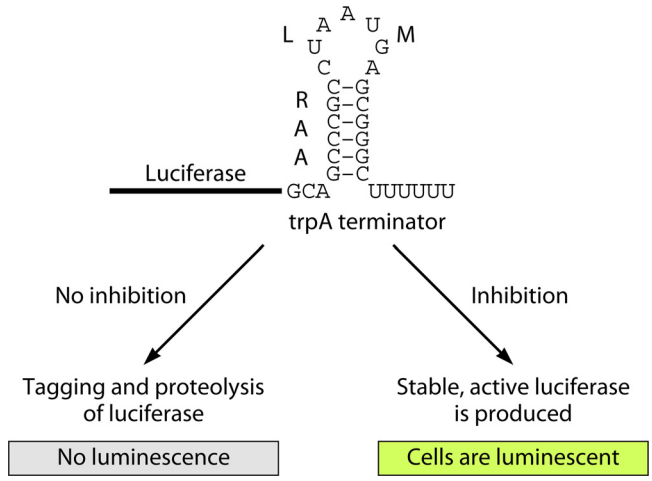

FIG 4 High-throughput screening assay to identify *trans*-translation inhibitors. The reporter contains a gene encoding luciferase with a strong transcriptional terminator inserted before the stop codon, such that transcription results in a nonstop mRNA. *E. coli* cells containing the reporter were screened in high-throughput format to identify compounds that inhibit *trans*-translation. When no inhibitor is present, translation of the nonstop mRNA results in *trans*-translation followed by proteolysis of luciferase, and cells produce no luminescence. Conversely, active luciferase is produced when a *trans*-translation inhibitor is present, resulting in luminescence.

ulence in many pathogenic strains and is not found in metazoans. Therefore, compounds that specifically inhibit *trans*-translation and not translation are likely to be effective for treating infections and yet have low toxicity for host cells. Compounds that inhibit *trans*-translation should kill *M. tuberculosis*, *N. gonorrhoeae*, *S. flexneri*, *H. influenzae*, *S. aureus*, and other species in which *trans*-translation is essential and could also prevent infection by *S. enterica*, *Y. pestis*, *F. tularensis*, *S. pneumoniae*, and other species that require *trans*-translation for virulence. Compounds that inhibit ArfA and ArfB in addition to the effect of *trans*-translation may have antibacterial activity against all species.

Several cell-based assays for *trans*-translation activity have been described, and they all have the same basic construction (91). A strong transcriptional terminator is inserted before the stop codon of a reporter gene, such as the *luc* gene encoding luciferase (Fig. 4). Because the reporter protein is made from a nonstop mRNA, the protein is tagged and degraded if there is no inhibitor present. In the presence of an inhibitor, active reporter protein is produced. In principle, such assays could be used for screening any compound library for inhibitors.

The results of one high-throughput screening (HTS) investigation of inhibitors of *trans*-translation have been reported (36, 91). Several small molecules identified by HTS inhibit *trans*-translation but not translation *in vitro*. Growth inhibition assays with these compounds showed that they have broad-spectrum antibacterial activity (91). One compound, KKL-35, has a MIC of <2 μg/ml against pathogenic strains of *F. tularensis*, *Y. pestis*, *B. anthracis*, *Burkholderia mallei*, and *S. aureus* (K. Keiler, unpublished data). For KKL-35, growth inhibition of *E. coli* was antagonized by low concentrations of puromycin, a drug that can release nonstop translation complexes by hydrolyzing peptidyl-tRNA on the ribosome (91). Likewise, growth inhibition of *S. flexneri* was antagonized by overexpression of *E. coli* ArfA. These results suggest that KKL-35 inhibits growth by preventing release of nonstop translation complexes. Although many additional tests are required to determine if KKL-35 can be developed into a new antibiotic, it is clear that *trans*-translation and alternate pathways to resolve nonstop translation complexes are druggable. These pathways should be considered a prime target for further antibiotic development.

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